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BURDEN OF MULTI-DRUG RESISTANCE IN *SALMONELLA TYPHI* ISOLATES OF ENTERIC FEVER.

DR. MAHNOOR WAHID, MBBS
NISHTAR HOSPITAL, MULTAN, PAKISTAN.

DR. HIRA AWAN, MBBS
WMO, DISTRICT HEADQUARTERS HOSPITAL, KHANEWAL, PAKISTAN.

DR. KHIZRA MUSSADIQ, MBBS
NISHTAR HOSPITAL, MULTAN, PAKISTAN.

Abstract;
Objective; To determine burden of multi-drug resistance among *Salmonella typhi* isolates of enteric fever at a tertiary care hospital. **Material and methods;** Consecutive 161 children with enteric fever were included in this study. This cross – sectional study was done at Department of Pediatric Medicine, Nishtar Medical University and Hospital, Multan from June 2017 to December 2018. Venous blood sample (3-5 ml) was drawn and it was immediately transported to Microbiology laboratory of Pathology Department, Nishtar Hospital Multan for blood culture and sensitivity test. Antibiotic drug resistance was determined after measuring diameter of zone of inhibition of a specific drug on Salmonella isolates on Mueller Hinton agar plates by modified Kirby baur disc diffusion method. Coated tongue, pain abdomen, anorexia and headache was assessed clinically. All the data were entered and analyzed using SPSS-11. **Results;** Of these 161 study cases, 91 (56.5%) were boys and 70 (43.5%) were girls. Mean age of our study cases was 5.79 ± 2.45 years (with minim age was 2 years while 12 years was maximum age). Previous history of use of antibiotics before 48 hours of the culture and sensitivity test was present in 93 (57.8%) of our study cases. Majority of our study cases having enteric fever presented with abdominal pain 65 (40.4%), headache 28 (17.4%) and diarrhea 17.4 % of our study cases. Chloramphenicol was sensitive in 40 (24.8%) of the *Salmonella typhi* isolates, Ampicillin was sensitive in 20 (12.4%), Co-trimoxazole in 20 (12.4%), Ciprofloxacin was sensitive in 88 (54.7%) isolates, Ofloxacin was sensitive in 101 (62.7%), Gentamycin was sensitive in 28 (17.4 %), Amoxycillin was sensitive in 36 (22.4 %). Multi – drug resistant strains were 71 (44%) in our study cases. **Conclusion;** Our study results have indicated that *S. typhi* isolates of enteric fever showed higher degree of antibiotic drug resistance. Multidrug resistant strains were more prevalent in our study which points towards well directed efforts of our health policy makers to adopt certain guidelines regarding use of these drugs in our population.
Keywords; Enteric fever, Multi drug resistance, Salmonella typhi.
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Introduction;

Typhoid fever is most commonly referred as severe systemic illnesses which are presenting with high fever and abdominal pain[1]. The main causative organisms culprit for this systemic illness may include S*almonell enterica* serotype *Typhi* which was previously known as S. typhi. Other serotypes of Salmonella such as S. enterica serotype paratyphi A, B, or C may also lead to similar type of syndromes a similar syndrome while it is very hard to predict culprit organism on the basis of clinical judgements [2]. Both typhoid fever and paratyphoid fever are commonly referred in a collective term as enteric fever. Salmonella is an important cause of febrile illnesses in over-crowded and impoverished populations having no or poor sanitation facilities, consuming unsafe water and foods and it is human restricted pathogen which is transmitted through feco-oral route[3]. The Global estimates of occurrence of Enteric fever is twenty one million cases causing 216500 deaths every year[4, 5]. Pakistan harbors high burden of the enteric fever showing an annual rate of 413/100, 000 persons per year while more than 90 % of worldwide episodes occur only in Asian countries. However SAARC region countries have

approximately rate of 110 cases/100, 000 persons every year. No non-human vector of Salmonella typhi has been reported yet and inoculums as small as 100,000 causative agents may cause illness in about 50% of healthy subjects. According to WHO, total incidence of enteric fever in Pakistan is almost 412 cases per 100, 000 persons every year [6, 7].

Because of the ready availability of the over the counter antibiotics and subsequent resistance to these drugs in areas of endemicity, enter fever is harder to treat as previously Chloramphenicol was used to treat this infection but in 1980 emergence of resistance limited its use. This was followed by emergence of multidrug resistant strains (combined resistance to chloramphenicol, ampicillin and cotrimoxazole) which was initially reported from India, Pakistan, Middle East and then from rest of the world[6]. Widespread use of fluroquinolones has led to the emergence of reduced susceptibility to the fluroquinolones[8]**.** Chloramphenicol was 56% resistant, Ampicillin was 67%, Cotrimoxazol 48% resistant, while Ciprofloxacin, Ceftriaxone and Ofloxacin were totally sensitive in a study conducted in children with enteric fever residing in slum areas of Karachi[9]. In a study from Nepal, Gentamycin was 82% resistant while Co-trimoxazole was 85% resistant, chloramphenicol and amoxicillin each were 63% resistant [10]. A study from India has reported Ampicillin 74.2%, Co-trimoxazole 69%, Chloramphenicol 67%, Amoxicillin 71%, Gentamycin 9.7%, Ciprofloxacin 26% and Ofloxacin 7.2% resistance among Salmonella typhi isolated from blood samples of children having enteric fever[5].

So we conducted this study to see pattern of antimicrobial drug resistance in Salmonella typhii isolates of enteric fever.

Material and Methods;

Consecutive 161 children with enteric fever were included in this study. Sample size was calculated using WHO sample size calculator anticipating 7.2 % drug resistance of Salmonella against Ofloxacin at 95 % confidence interval. Patients having typhidot IgM test positive were included in this study. This study was done at Department of Pediatric Medicine, Nishtar Medical University and Hospital, Multan from June 2015 to December 2016. All the patients aged 2-12 years of either sex having typhidot IgM positive patients presenting with fever $\geq$ 101 F^0 plus any one of the followings such as abdominal pain, coated tongue, splenomegaly, constipation, diarrhea, hepatomegaly, anorexia and headache; were included in this cross – sectional study. Patients having typhidot positive cases which are already taking antibiotics within 48 hours, patients with perforations, shock or seizures, patients with congenital or acquired immunodeficiency and those with poor oral intake were excluded from our study. Brief history regarding the clinical presentations such as fever, Headache, Constipation, Diarrhea, Anorexia, Pain abdomen, Hepatomegaly, Splenomegaly and coated tongue and demographics were recorded. Venous blood sample (3-5 ml) was drawn and it was immediately transported to Microbiology laboratory of Pathology Department, Nishtar Hospital Multan for blood culture and sensitivity test. Any growth observed, after 24 hours was subjected to Drug sensitivity/resistance test using modified Kirby baur disc diffusion method on a Mueller Hinton agar plates. Antibiotic drug resistance was determined after measuring diameter of zone of inhibition of a specific drug on Salmonella isolates on Mueller Hinton agar plates by modified Kirby baur disc diffusion method. Coated tongue, pain abdomen, anorexia and headache was assessed clinically. Fever ($\geq$ 101 F^0) was assessed by thermometer. Diarrhea was characterized by 3 watery stools in 24 hours time. Hepatomegaly and splenomegaly were also assessed clinically. All the data were entered and analyzed using SPSS-11. Descriptive statistics was applied to calculate mean and standard deviation for the age of the patients. Frequencies and percentage were calculated for the categorical variables like presenting complaints (such as fever, headache, constipation, diarrhea, pain abdomen, hepatomegaly, anorexia coated tongue and splenomegaly) and antibiotic drugs (such as Chloramphenicol, Ampicillin, Co-trimoxazole, Amoxycillin, Ciprofloxacin, Ofloxacin and Gentamycin).

Results;

A total of 161 children suffering from enteric fever with positive isolates of *Salmonella typhi* meeting inclusion and exclusion criteria of this study were included in this study. Of these 161 study cases, 91 (56.5%) were boys and 70 (43.5%) were girls. Mean age of our study cases was 5.79 ± 2.45 years (range; 2 – 12 years). Mean age of boys was 5.47 ± 2.14 years while those of girls was 6.20 ± 2.75 years (p=0.062) while 104 (64.6%) were in the age group of 2 – 7 years. Majority of our study cases having enteric fever presented with abdominal pain 65 (40.4%), headache 28 (17.4%) and diarrhea 17.4 % of our study cases. Chloramphenicol was sensitive in 40 (24.8%) of the *Salmonella typhi* isolates, Ampicillin was sensitive in 20 (12.4%), Co-trimoxazole in 20 (12.4%), Ciprofloxacin was sensitive in 88 (54.7%) isolates, Ofloxacin was sensitive in 101 (62.7%),

Gentamycin was sensitive in 28 (17.4 %), Amoxycillin was sensitive in 36 (22.4 %). Multi – drug resistant strains were 71 (44%) in our study cases.

Discussion;

Enteric fever, particularly in underdeveloped countries, is a public health issue. It is a major health problem in tropical regions including Pakistan. Recent emergence of antimicrobial resistance among causative agent (*Salmonella typhii)* has given rise to some serious concerns in our society as antibiotic drugs provide most effective treatment options in enteric fever [11, 12]. Irrational use of these drugs, readily availability without physician's prescription have compounded in the emergence of resistance in drugs which were effective against *Salmonella*.

Different studies have reported male gender predominance in children with enteric fever. Our study results are also in compliance with these findings. Of these 161 study cases, 91 (56.5%) were boys and 70 (43.5%) were girls. Hussain et al [13] also reported male gender predominance in their study i.e. 62 % which is close to our study results. Iqbal et al [14] reported 63 % boys having enteric fever which is again in compliance with that of our study results. Lakhany et al [15] from Karachi reported male to female ratio being 2:1 which is similar to our findings and Fazil et al [16] reported the same.

Mean age of our study cases was 5.79 ± 2.45 years (range; 2 – 12 years). Mean age of boys was 5.47 ± 2.14 years while those of girls was 6.20 ± 2.75 years (p=0.062) while 104 (64.6%) were in the age group of 2 – 7 years. Fazil et al [16] reported 5 ± 3 years age of children with enteric fever which is close to our study results. Khurshid et al [17] reported 6 years mean age of the children with enteric fever these findings are close to our study results. Similar results have been reported by Lakhany et al from Karachi [15] and Iqal et al from Lahore [14].

Majority of our study cases having enteric fever presented with abdominal pain 65 (40.4%), headache 28 (17.4%) and diarrhea 17.4 % of our study cases. Similar results have been reported by Lakhany et al from Karachi. [15] A study conducted by Rasaily et al reported headache (36.0%), chill and rigor (23.2%), diarrhea (37.2%), anorexia (26.2%), vomiting (23.8%), cough (18.0%) and abdominal pain (19.8%) [18].

Chloramphenicol has been drug of first choice since its introduction in 1948 and is being widely used to treat patients with enteric fever. Ampicillin and cotrimoxazole are also used as an alternative therapy against *S.* Typhi but emergence of drug resistance against these drugs (Multidrug resistant *S. typhi)* have raised serious concerns to treat these infections particularly in South Asian countries. Chloramphenicol was sensitive in 40 (24.8%) of the *Salmonella typhi* isolates and was resistant in 47.8% of isolates. Mathura et al from Nepal reported 63 % resistance with this drug. Khan et al [9] reported Chloramphenicol 56% resistant, Similar findings have been reported by Imran et al [19] which are close to our findings. In our study, Ampicillin was sensitive in 20 (12.4%) and resistant in 62.1 % of the *salmonella* isolates while a study conducted by Javed et al [20] reported 23% sensitivity to this drug this decrease in sensitivity in our study points towards increasing drug resistance trends in our population. While khan et al[9] reported 67 % resistance, which is again consistent with previous findings. Kumar et al[5] reported 75% resistance with this drug, these findings are similar to our study results. In our study, Co-trimoxazole was sensitive in 20 (12.4%) and resistant in 67.7 % of the isolates while Khan et al[9] 48 % resistance which also points towards increasing trends of drug resistance in our population. Mathura et al[10] reported 85 % resistance with Co-trimoxazole which close to our study results. In our study, Ciprofloxacin was sensitive in 88 (54.7%) and was resistant in 17.4 % of the isolates. Khan et al[9] reported ciprofloxacin was 100% sensitive in children with enteric fever while Javed et al [20] reported 86% sensitivity again showing increasing trends towards drug resistance in our population. In India Ciprofloxacin was resistant in 26 % children with enteric fever which is close to our study results. In our study, Ofloxacin was sensitive in 101 (62.7%) and resistant in 27.3 % isolates while Kumar et al [5] reported 7.2 % drug resistance from India which points towards resistant strains in our population. In our study, Gentamycin was sensitive in 28 (17.4 %) and resistant in 72.7 % of the isolates. Javed et al reported [20] 90% sensitivity which is much higher than our study findings. Mathura et al[10] from Nepal reported 82% sensitivity of the Gentamycine. In our study, Amoxicillin was sensitive in 36 (22.4 %) and resistant in 57.8 % of the isolates. Mathura et al[10] reported 63% resistance in this drug which is close to our finding while Amoxicillin was totally sensitive as reported by Javed et al [20]. Kumar et al[5] from India reported 71 % resistance with Amoxicillin which is close to our study results.

Conclusion;

Our study results have indicated that *S. typhi* isolates of enteric fever showed higher degree of antibiotic drug resistance. Multidrug resistant strains were more prevalent in our study which points towards well directed efforts of our health policy makers to adopt certain guidelines regarding use of these drugs in our population. Irrational use of antibiotic drugs followed by their easy availability and natural adaptation of these pathogens have led to increased drug resistance against those drugs which are commonly used to treat enteric fever. Only ciprofloxacin and Ofloxacin were found to be effective in our study. Burden of enteric fever can be substantially lowered by rapid diagnosis followed by appropriate antimicrobial drug treatment.

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